Performance of the BG1Luc and ER β-Lactamase Estrogen Receptor Transactivation Assays in Tox21 Compound Screening

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Introduction

- The mission of the U.S. Tox21 program is to research, develop, validate, and translate innovative chemical testing methods for the characterization of toxicity pathways. Tox21 integrates Federal resources and expertise from the following offices:
  - Environmental Protection Agency
  - National Institutes of Environmental Health Sciences/National Toxicology Program
  - National Institutes of Health (NIH)/NIH Center for Advancing Translational Sciences
  - Food and Drug Administration
- The use of robotics platforms to screen thousands of chemicals provides a cost-effective approach to prioritize further testing of potentially toxic chemicals. Exposure to "endocrine active chemicals" (EACs) may result in developmental or reproductive problems.
- EACs may affect growth and development through a variety of mechanisms. One such mechanism is estrogenic signaling.
- Estrogenic signaling pathways are well-characterized, and a number of test methods that target them have been developed. Two estrogen receptor (ER) transactivation assays, the BG1Luc4E2 (BG1Luc) and the HEK293 ER β-lactamase (ER-Bla), have been adapted to a high-throughput screening (HTS) platform and incorporated into the Tox21 program.

Table 1. Overview of Differences Between the Methods

	BG1Luc HTS	ER-Bla HTS
Cell Line	BG-1Luc4E2	HEK293
Tissue of Origin	Ovary	Kidney
Receptor Expression	Native	Stably transfected
Receptors	ER-α and ER-β	ER-α ligand binding domain
Response Element	Estrogen-response element	Upstream β-lactamase reporter gene activator sequence
Reporter	Luciferase	β-Lactamase
Viability Detection	Fluorescent	Luminescent

Use of the Assays to Screen the Tox21 Chemicals

- The Tox21 10K chemical library was screened using both assays in agonist and antagonist modes.
- Cell viability was simultaneously evaluated in each assay to distinguish antagonism from cytotoxicity.
- Data quality was evaluated in several ways:
  - Computation of metrics including signal-to-background detection ratio, coefficient of variation, and Z' factor (Zhang 1999)
  - Comparison to reference standard values
  - Comparison of 88 chemicals duplicated on every test plate (intra-assay)
  - Comparison of outcome matches across three runs (inter-assay)

Table 2. Agonist Data Quality

		BG1Luc HTS[a]	ER-Bla HTS[a]
Signal-to-background and Z' factor	Signal-to-background ratio	2.5 ± 0.3	4.6 ± 0.6
	Coefficient of variation (%)	10.3 ± 5.9	4.7 ± 3.7
	Z' factor	0.5 ± 0.25	0.53 ± 0.09
Reference Standard Values	Estradiol EC_{50} (pM)[b]	30 ± 70	275 ± 80
Intra-assay	EC_{50} correlations (R^2)[c]	0.80	0.83
Inter-assay	Active match (%)	16	7
	Inactive match (%)	87	71
	Fold difference in AC_{50} among three experiments[d]	1.5	1.4

Abbreviation: HTS = high-throughput screening.

[a] All values are reported as mean values. Standard deviation is reported where applicable.

[b] EC_{50} is the half-maximal effective concentration.

[c] Intra-assay R^2 values were calculated for all positive test substances.

[d] AC_{50} is the half-maximal activity concentration (Inglese 2006).

Table 3. Antagonist Data Quality

		BG1Luc HTS[a]	ER-Bla HTS[a]
Signal-to-background and Z' factor	Signal-to-background ratio	8.0 ± 0.9	3.3 ± 0.8
	Coefficient of variation (%)	6.5 ± 2.8	5.1 ± 2.8
	Z' factor	0.8 ± 0.07	0.4 ± 0.1
Reference Standard Values	4-Hydroxytamoxifen IC_{50} (nM)[b]	70.8 ± 12.4	5.8 ± 3.8
Intra-assay	IC_{50} correlations (R^2)[c]	0.76	0.47
Inter-assay	Active match (%)	12	10
	Inactive match (%)	80	78
	Fold difference in AC_{50} among three experiments[d]	1.5	1.5

Abbreviation: HTS = high-throughput screening.

[a] All values are reported as mean values. Standard deviation is reported where applicable.

[b] IC_{50} is the half-maximal inhibitory concentration.

[c] Intra-assay R^2 values were calculated for all positive test substances.

[d] AC_{50} is the half-maximal activity concentration (Inglese 2006).

Comparison to ICCVAM Performance Standards

- The U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods coordinated an international validation study of the BG1Luc assay for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).
- A test method evaluation report summarizing the study (ICCVAM 2011) contained performance standards for developing functionally and mechanistically similar test methods and for demonstrating proficiency in the BG1Luc assay. The performance standards specify reference substances for both agonist and antagonist modes with expected positive and negative outcomes for each substance.
- HTS data for test chemicals were reviewed and classified as positive, negative, or inconclusive. For a test substance to be classified as positive, it needed to have a response greater than or equal to 20% that of the positive control and have a semi-sigmoidal response curve.
- Results obtained in the BG1Luc HTS and ER-Bla HTS assays were compared to outcomes specified in the performance standards (**Tables 4–7**).
- Discordant results are detailed in **Figure 1.**

Agonist Sensitivity and Specificity

Table 4. BG1Luc HTS and ER-Bla HTS Agonist Results Compared to BG1Luc Manual Performance Standards

PS Substances	CAS RN	Classification		
		PS	BG1Luc HTS	ER-Bla HTS
17-α Estradiol	57-91-0	POS	POS	POS
17-α Ethinyl estradiol	57-63-6	POS	POS	POS
17-β Estradiol	50-28-2	POS	POS	POS
19-Nortestosterone	434-22-0	POS	POS	POS
4-Cumylphenol	599-64-4	POS	POS	POS
4-*tert*-Octylphenol	140-66-9	POS	POS	POS
Apigenin	520-36-5	POS	POS	POS
Bisphenol A	80-05-7	POS	POS	POS
Bisphenol B	77-40-7	POS	POS	POS
Butylbenzyl phthalate	85-68-7	POS	POS	IC
Chrysin	480-40-0	POS	POS	POS
Coumestrol	479-13-0	POS	POS	IC
Daidzein	486-66-8	POS	POS	POS
Dicofol	115-32-2	POS	NEG	IC
Diethylstilbestrol	56-53-1	POS	POS	POS
Estrone	53-16-7	POS	POS	POS
Ethyl paraben	120-47-8	POS	IC	NEG
Fenarimol	60168-88-9	POS	IC	IC
Genistein	446-72-0	POS	POS	POS
Kaempferol	520-18-3	POS	POS	POS
Kepone	143-50-0	POS	POS	POS
***meso*-Hexestrol**	84-16-2	POS	POS	POS
Methyl testosterone	58-18-4	POS	POS	POS
Norethynodrel	68-23-5	POS	POS	POS
***o.p'*-DDT**	789-02-6	POS	POS	POS
***p-n*-Nonylphenol**	104-40-5	POS	POS	NEG
***p.p'*-Methoxychlor**	72-43-5	POS	POS	NEG
Atrazine	1912-24-9	NEG	NEG	NEG
Bicalutamide	90357-06-5	NEG	NEG	NEG
Corticosterone	50-22-6	NEG	NEG	NEG
Hydroxyflutamide	52806-53-8	NEG	NEG	NEG
Linuron	330-55-2	NEG	NEG	NEG
Phenobarbital	50-06-6	NEG	NEG	NEG

Abbreviations: CAS RN = Chemical Abstracts Service Registry Number®; HTS = high-throughput screening; IC = inconclusive; NEG = negative; POS = positive; PS = performance standards.

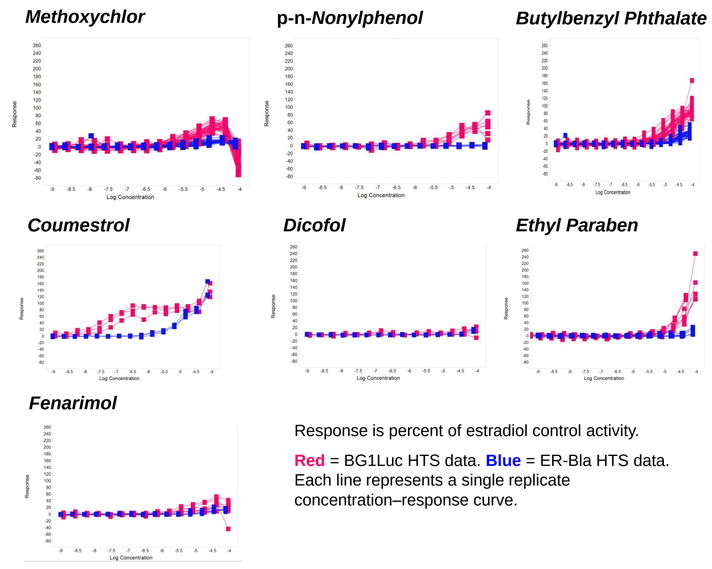
Table 5. Agonist Sensitivity and Specificity for the BG1Luc and ER-Bla Assays

	BG1Luc HTS	ER-Bla HTS
Sensitivity	96% (24/25)	87% (20/23)
Specificity	100% (7/7)	100% (7/7)
Accuracy	97% (31/32)[a]	90% (27/30)[a]

Abbreviation: HTS = high-throughput screening.

[a] Of the 34 agonist substances in the performance standards, two were omitted in BG1Luc HTS and four were omitted in ER-Bla HTS because the results were inconclusive.

Figure 1. Substances With Discordant Results in Agonist Assays



Response is percent of estradiol control activity.

Red = BG1Luc HTS data. **Blue** = ER-Bla HTS data. Each line represents a single replicate concentration–response curve.

Antagonist Sensitivity and Specificity

- Expected positive and negative outcomes from the ICCVAM performance standards are compared to observed outcomes in the BG1Luc HTS and ER-Bla HTS assays in **Tables 6** and **7**.
- None of the outcomes for either the BG1Luc HTS or the ER-Bla HTS assay was discordant with the performance standards or with the other assay, although four substances yielded inconclusive results with the ER-Bla HTS.

Table 6. BG1Luc HTS and ER-Bla HTS Antagonist Results Compared to BG1 Manual Performance Standards

PS Substances	CAS RN	Classification		
		PS	BG1 HTS	ER-Bla HTS
4-Hydroxytamoxifen	68047-06-3	POS	POS	POS
Raloxifene HCl	82640-04-8	POS	POS	POS
Tamoxifen	10540-29-1	POS	POS	POS
17-α Ethinyl estradiol	57-63-6	NEG	NEG	NEG
Apigenin	520-36-5	NEG	NEG	IC
Chrysin	480-40-0	NEG	NEG	NEG
Coumestrol	479-13-0	NEG	NEG	NEG
Genistein	446-72-0	NEG	NEG	IC
Kaempferol	520-18-3	NEG	NEG	IC
Resveratrol	501-36-0	NEG	NEG	IC

Abbreviations: CAS RN = Chemical Abstracts Service Registry Number®; HTS = high-throughput screening; IC = inconclusive; NEG = negative; POS = positive.

Table 7. Antagonist Sensitivity and Specificity for the BG1Luc HTS and ER-Bla HTS Assays

	BG1Luc HTS	ER-Bla HTS
Sensitivity	100% (3/3)	100% (3/3)
Specificity	100% (7/7)	100% (3/3)
Accuracy	100% (10/10)	100% (6/6)[a]

Abbreviation: HTS = high-throughput screening.

[a] Of the 10 agonist substances in the performance standards, four were omitted in ER-Bla HTS because the results were inconclusive.

References

A reference list for this poster is available at http://ntp.niehs.nih.gov/iccvam/meetings/9wc/casey-bg1bla-refs.pdf

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A summary of NICEATM and ICCVAM activities at the Ninth World Congress is available on the National Toxicology Program website at http://ntp.niehs.nih.gov/go/41583.